CLAIMS

We Claim:

1. A process for generating a complementary DNA (cDNA) molecule from an RNA molecule comprising:

   a) annealing a first primer containing a non-replicable element, with or without a cleavable element, to an RNA molecule,

   b) generating a first strand cDNA product,

   c) annealing a second primer containing a non-replicable element, with or without a cleavable element, to the first strand cDNA product, and

   d) generating a second strand cDNA product.

2. A process for amplifying a cDNA molecule comprising:

   a) generating a cDNA molecule according to the process of claim 1;

   b) combining the first and second cDNA strands in a reaction mixture with a primer containing a non-replicable element and/or a cleavable element, under conditions such that first generation primer extension products are produced using said strands as templates, and wherein the primer for the first strand is selected such that a first generation primer extension product produced using the first strand as a template, when separated from the first strand, can serve as a template for

synthesis of a second generation primer extension product of the primer for the second strand;

c) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and

d) treating the first generation primer extension products with the primers of step (b) under conditions such that second generation primer extension products are produced using the first generation primer extension products as templates, wherein the second generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of interest and no more than an insufficient portion of the binding site for said first primers for producing said first generation primer extension products.

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3. A process for amplifying a cDNA molecule comprising:

(a) generating a cDNA molecule according to the process of claim 1;

(b) combining the first and second cDNA strands in a reaction mixture with first primers and second primers, each of said first and second primers containing a non-replicable element and/or a cleavable element, under conditions such that a first generation primer extension product is synthesized using said strands as templates, and wherein the first and second primers are selected such that the first generation primer extension products, when separated from

their templates, can serve as templates for synthesis of second generation primer extension products of the first and second primers;

(c) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and

(d) treating the first generation primer extension products with the first and second primers under conditions such that second generation primer extension products are synthesized using the first generation primer extension products as templates, wherein the second generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of interest and no more than an insufficient portion of the binding site for said first and second primers for producing said first generation primer extension products.

4. A process for amplifying a cDNA molecule comprising:

(a) generating a cDNA molecule according to the process of claim 1;

(b) combining the first and second cDNA strands in a reaction mixture with a series of nested primers, each primer containing a non-replicable element and/or a cleavable element, said series of nested primers comprising a plurality of primers which are complementary to different portions of said strands and are 5' to one another with respect to said strand and which do not overlap with one another at the position of said non-replicable element or cleavable element;

(c) subjecting said reaction mixture to conditions such that first generation primer extension products are produced from first primers, and not from other of said series of nested primers using the first and second strands as templates, wherein said first primers are primers of said nested primers which are most 3' with respect to said sequence of interest, and wherein the first primers are selected such that a first generation primer extension product from this step, when separated from its template, can serve as a template for synthesis of a second generation extension product of the first primer for the complement of said strand;

(d) separating the first generation primer extension products from their respective templates to produce single-stranded molecules;

(e) exposing said reaction mixture to conditions such that second generation primer extension products are generated by said first primers using first generation primer extension products as templates, wherein the second generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of interest and no more than an insufficient portion of the binding site for said first primers for producing said first generation primer extension products;

(f) separating the second generation primer extension products from their template to produce single stranded molecules;

(g) subjecting the reaction mixture of step (f) to reaction conditions such that next generation primer extension products are synthesized from another primer of said series of nested primers using second generation primer extension products as templates, and separating the thus produced next generation primer extension products from their templates to produce single-stranded molecules; and

(h) repeating step (g) such that each repeat of the step comprises subjecting the reaction mixture to conditions such that next generation primer extension products are synthesized from a different primer of said series of nested primers using the next prior generation primer extension products as templates.

5. A process for amplifying a cDNA molecule comprising:

(a) generating a cDNA molecule according to the process of claims 1;

(b) combining the first and second strands in a reaction mixture with a series of nested primes, each primer containing a non-replicable element and/or a cleavable element, said series of nested primers comprising a plurality of primers which are complementary to different portions of said strands and flank the sequence of interest but do not overlap with one another at the position of said non-replicable element or cleavable element;

(c) subjecting said reaction mixture to conditions whereby each of said primers is capable of binding to its respective complementary site;

(d) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and

(e) repeating steps (c) and (d) whereby next generation primer extension products are synthesized from a different primer of said series of nested primers using the next prior generation primer extension products as templates.

6. The process according to any one of claims 1-5, wherein the non-replicable element is not located at the terminal residue of any of said primers.

7. The process according to any one of claims 1-5, wherein the cleavable element is not located at the terminal residue of any of said primers

8. The process of any one of claims 2-5, wherein said primer contains a non-replicable element.

9. The process of any one of claims 2-5, wherein said primer contains a cleavable element.

10. The process of claim 9 wherein the cleavable element is a ribonucleoside.

11 The process of claim 10 wherein said first generation primer extension product is cleaved by treating said product with ribonuclease.

12. The process according to any one of claims 1-5 wherein the non-replicable element is a derivative of a deoxyribonucleotide.

13. The process according to any one of claims 1-5 wherein the non- replicable element is a derivative of a ribonucleotide.

14. The process according to claim 13 wherein the non- replicable element is a residue of 1,3-propane diol.

15. The process according to claim 13 wherein the non- replicable element is a residue of 1,4-anhydro-2-deoxy-D- ribitol.

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16. A process for generating a complementary DNA (cDNA) molecule from an RNA molecule comprising:

a) annealing a primer containing a non-replicable element, with or without a cleavable element, to an RNA molecule, and

b) generating a first strand cDNA product.

17. A process for amplifying a cDNA molecule comprising:

a) generating a cDNA molecule according to the process of claim 16;

b) combining the cDNA strand in a reaction mixture with a primer containing a non-replicable element and/or a cleavable element, under conditions such that first generation primer extension products are produced using said strands as templates, and wherein the primer for the first strand is selected such that a first generation primer extension product produced using the first strand as a template, when separated from the first strand, can serve as a template for synthesis of a second generation primer extension product of the primer for the second strand;

c) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and

d) treating the first generation primer extension products with the primers of step (b) under conditions such that second generation primer extension products are produced using the first generation primer extension products as templates, wherein the second generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of interest and no more than an insufficient portion of the binding site for said first primers for producing said first generation primer extension products.

18. A process for amplifying a cDNA molecule comprising:

a) generating a cDNA molecule according to the process of claim 16;

b) combining the first and second cDNA strands in a reaction mixture with first primers and second primers, each of said first and second primers containing a non-replicable element and/or a cleavable element, under conditions such that a first generation primer extension product is synthesized using said strands as templates, and wherein the first and second primers are selected such that the first generation primer extension products, when separated from their templates, can serve as templates for synthesis of second generation primer extension products of the first and second primers;

c) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and

d) treating the first generation primer extension products with the first and second primers under conditions such that second generation primer extension products are synthesized using the first generation primer extension products as templates, wherein the second generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of interest and no more than an insufficient portion of the binding site for said first and second primers for producing said first generation primer extension products.

19. A process for amplifying a cDNA molecule comprising:

a) generating a cDNA molecule according to the process of claim 16;

b) combining the first and second cDNA strands in a reaction mixture with a series of nested primers, each primer containing a non-replicable element and/or a cleavable element, said series of nested primers comprising a plurality of primers which are complementary to different portions of said strands and are 5' to one another with respect to said strand and which do not overlap with one another at the position of said non-replicable element or cleavable element;

c) subjecting said reaction mixture to conditions such that first generation primer extension products are produced from first primers, and not from other of said series of nested primers using the first and second strands as templates, wherein said first primers are primers of said nested primers which are most

3' with respect to said sequence of interest, and wherein the first primers are selected such that a first generation primer extension product from this step, when separated from its template, can serve as a template for synthesis of a second generation extension product of the first primer for the complement of said strand;

d) separating the first generation primer extension products from their respective templates to produce single-stranded molecules;

e) exposing said reaction mixture to conditions such that second generation primer extension products are generated by said first primers using first generation primer extension products as templates, wherein the second generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of interest and no more than an insufficient portion of the binding site for said first primers for producing said first generation primer extension products;

f) separating the second generation primer extension products from their template to produce single stranded molecules;

g) subjecting the reaction mixture of step (f) to reaction conditions such that next generation primer extension products are synthesized from another primer of said series of nested primers using second generation primer extension products as templates, and separating the thus produced next generation

primer extension products from their templates to produce single-stranded molecules; and

h) repeating step (g) such that each repeat of the step comprises subjecting the reaction mixture to conditions such that next generation primer extension products are synthesized from a different primer of said series of nested primers using the next prior generation primer extension products as templates.

20. A process for amplifying a cDNA molecule comprising:

a) generating a cDNA molecule according to the process of claims 16;

b) combining the first and second strands in a reaction mixture with a series of nested primes, each primer containing a non-replicable element and/or a cleavable element, said series of nested primers comprising a plurality of primers which are complementary to different portions of said strands and flank the sequence of interest but do not overlap with one another at the position of said non-replicable element or cleavable element;

c) subjecting said reaction mixture to conditions whereby each of said primers is capable of binding to its respective complementary site;

d) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and

e) repeating steps (c) and (d) whereby next generation primer extension products are synthesized from a different primer of said series of nested

primers using the next prior generation primer extension products as templates.

21. The process according to any one of claims 16-20, wherein the non-replicable element is not located at the terminal residue of any of said primers.

22. The process according to any one of claims 16-20, wherein the cleavable element is not located at the terminal residue of any of said primers

23. The process of any one of claims 17-20, wherein said primer contains a non-replicable element.

24. The process of any one of claims 17-20, wherein said primer contains a cleavable element.

25. The process of claim 24 wherein the cleavable element is a ribonucleoside.

26. The process of claim 25 wherein said first generation primer extension product is cleaved by treating said product with ribonuclease.

27. The process according to any one of claims 16-20 wherein the non-replicable element is a derivative of a deoxyribonucleotide.

28. The process according to any one of claims 16-20 wherein the non-replicable element is a derivative of a ribonucleotide.

29. The process according to claim 28 wherein the non- replicable element is a residue of 1,3-propane diol.

30. The process according to claim 28 wherein the non- replicable element is a residue of 1,4-anhydro-2-deoxy-D- ribitol.